REMARKS

Claims 1-34 are pending in the application.

Claims 10, 23 and 31 are each amended above in a similar manner that causes the claims to be patentable over the prior art.

No new matter has been added to the application by these specification and claim amendments.

I. THE ALLOWED CLAIMS

On page 1, in the Office Action Summary, Applicants note that claims 1-9, 11 - 22, 24 – 30 and 32 - 34 are allowable, and this is very much appreciated.

The Applicant assumes that reference on page 6 line 1 to only claims 1-9, 11, 17-22, 24 and 32 being allowable is a typographical error, because only claims 10, 23 and 31 are identified as being rejected over the prior art.

II. THE OBVIOUSNESS REJECTION

Claims 10, 23 and 31 stand rejected for obviousness over Vaisberg Eu)(WO 02/47032) in view of Sunblad et al.

Claims 10, 23 and 31 have been amended in a like manner to overcome the 35 U.S.C. 103(a) objection. The amendments will be discussed in relation to claim 10 although identical remarks apply to claims 23 and 31.

Claim 10 now reads as follows:

10. A method of measuring mitotic activity from histopathological specimen color image data, the method having the steps of:

a) staining a histopathological specimen with a staining agent to color and delineate tissue and cellular structure appropriately for assessment of mitotic activity,

b) obtaining color image data from the stained histopathological specimen,

c) measuring an intensity profile of an image region corresponding to a potentially mitotic figure, ~~and~~

d) counting the image region as indicating a mitotic figure if its intensity profile has a ~~value~~ non-zero number of pixels with intensity associated with mitotic figure imagery, and that pixel number is greater than a prearranged threshold value, ~~at a position in the profile having intensity associated with mitotic figure imagery.~~

e) if one or more other image regions corresponding to potentially mitotic figures are available in the specimen image, repeating steps c) and d) for such region or regions,

f) repeating steps c), d) and e) for additional histopathological specimen image data in order to obtain mitotic figure counts for a plurality of specimen images, and

g) summing the mitotic figure counts obtained in steps c) to f) to provide an indication of degree of mitotic activity.

As compared to the previous version of claim 10, the new version is limited broadly speaking to color image data, to counting an image region as mitotic if its profile has a non-zero number of pixels greater than a prearranged threshold and with intensity associated with mitotic figure imagery, and to iterating the procedure for other image regions in the specimen image data and for other specimen image data. It is based on Applicants' specification at page 25 line 27 to page 29 last line, particularly page 28 line 27 to page 29 last line.

In rejecting claims 10, 23 and 31 for obviousness, the Examiner relies upon Vaisberg for disclosing a method of measuring mitotic activity from histopathological specimen image data, the method having the steps of:

a) measuring an intensity profile of an image region corresponding to a potentially mitotic figure;

b) counting the image region as indicating a mitotic figure if its profile has a value greater than a prearranged threshold at a position in the profile having intensity associated with mitotic figure imagery.

Applicants have amended claim 10 and claims 23 and 31 above to distinguish them more clearly over the cited prior art. In particular, claim 10 is now limited *inter alia* to staining a histopathological specimen with a staining agent to color and delineate tissue and cellular structure appropriately for assessment of mitotic activity, obtaining color image data from the staining of the stained histopathological specimen, and measuring an intensity profile of an image region in the color image data, the image region corresponding to a potentially mitotic figure.

A first reason why claims 10, 23 and 31 are non-obvious is because the cited prior art, and in particular Vaisberg does not disclose histopathological specimen image data, i.e. image data from a histopathological specimen in the form of a section of

human tissue cut from a patient in a biopsy operation and stained with a chemical to highlight mitotic cells. Moreover, Vaisberg does not disclose obtaining color image data from the staining, or measuring an intensity profile of an image region in such data as presently claimed.

Vaisberg does disclose cultured human lung cancer cells (page 29 lines 7-8), placed in a liquid suspension (page 29 lines 8-12), and treated with a fluorescent DNA indicator before being imaged (page 29 lines 15-16). Vaisberg's cultured human lung cancer cells are not, however, histopathological specimens. Therefore what Applicant is saying is that Vaisberg does not disclose histopathological specimen image data, i.e. image data from a histopathological specimen. A histopathological specimen is a slice or section of human tissue cut from a patient in a biopsy operation: it is mounted upon a histopathology slide and stained with a chemical to highlight features of interest - mitotic cells in the present case. Moreover, Vaisberg's cultured human lung cancer cells are not stained to show mitosis by reflection of incident light as in conventional histopathology. Instead, they are treated to indicate DNA by radiation emission, i.e. fluorescence etc. (e.g. page 11 line 19).

On page 4, lines 23-34, Vaisberg discloses obtaining mitosis indicator parameters by treating a cell with an agent that selectively associates with DNA and emits a signal recorded as a location of DNA within the cell. Workable mitosis indicator parameters include variance in DNA concentration within the cell, the size of a region of DNA within the cell, and a maximal concentration of DNA within the cell. A specific set of parameters includes the average DNA concentration values and the area occupied by DNA. This method of measuring mitosis is quite different to specimen preparation in conventional histopathology to measure mitosis.

The Examiner alleges that Vaisberg discloses (page 3, lines 7-8) "analyze images of cells and categorize the cells in particular cell cycle phases based upon certain features". This is true. However, the images are not histopathology images and the features are not histopathology image features for the reasons noted immediately above. Moreover Vaisberg indicates DNA by emission of radiation, i.e. fluorescence etc. (e.g. page 11 line 19), not reflection of incident light. The data yielded by Vaisberg

is therefore not histopathological specimen image data as claimed in claims 10, 23 and 31.

The Examiner states that page 3, lines 9-27 of Vaisberg discloses "characterize a cell as mitotic based on morphological and textual parameter such as pixel intensities". The examiner's position in with respect to this claim element is wrong. The relied upon extract from Vaisberg actually reads "characterize a cell as mitotic or interphase based on morphological and textual parameters such as the variance of the pixel intensities" (the Examiner's omissions underlined). It is the complete unedited passage of Vaisberg that one skilled in the art would consider at the time of the invention. One skilled in the art would understand that this passage does not disclose determining mitosis directly from pixel intensity as the examiner maintains and instead would understand the passage to refer to indirectly determining mitosis from pixel intensity variance σ or standard deviation given by:

$$\sigma = \sqrt{\frac{\sum_{i=1}^{N}(I_i - I_{av})^2}{N-1}}$$

where N is the number of pixels, the ith pixel has intensity I_i, and I_{av} is the average intensity of the N pixels.

The Examiner also refers to page 12, lines 1-9 of Vaisberg for disclosing the claim feature of "pixels with intensity values above threshold in a given neighborhood are belong to a particular cell". The examiner's understanding of the cited Vaisberg extract is technically wrong. In fact the relevant extract from Vaisberg actually reads "pixels with intensity values above threshold in a given neighborhood are deemed to belong to a particular cell". (Examiner's omission underlined). Once again the examiner is paraphrasing the passage in hindsight with the Applicant's invention in mind. Actually, one skilled in the art would understand that the entire passage has nothing whatsoever to do with mitosis measurement. Instead the referenced threshold is used to discriminate between cell (DNA) images and background, with background being objects which are thought not to be cells. (See Vaisberg page 12, lines 7-8).

The Examiner acknowledges that Vaisberg does not explicitly disclose "staining a histopathological specimen with a staining agent to delineate tissue and cellular

structure appropriately for assessment of mitotic activity". The examiner attempts to overcome this deficiency by citing Sunblad as disclosing this claim feature. The Examiner refers to extracts from Sunblad as follows:

a) page 1750, right column, lines 4-9: after staining, removing the apical meristem from each bud and squashing it into a single cell layer preparation.

b) page 1752, left column, lines 16-29, Statistical analysis: PCA was first used to obtain an overview of differences and similarities between different cell cycle stages in terms of image analysis parameters *(in Table 1, Applicants' italics)*. Thereafter, PCA was used to construct a model based only on interphase nuclei, and used to differentiate between dividing and non-dividing cells. For all multivariate studies, 14 image analysis parameters were used. To minimize problems related to variations in illumination of samples under the microscope or variations in staining intensity, all parameters except for Area--Fract, were relative parameters, calculated from original image parameters according to Table 1. For PCA, the systematic variation in the data matrix x *(sic, X?)* composed of the variables (i.e. image parameters) and objects (i.e. nuclei) was described by the model:

$$x_{ik} = x_k + \sum_{a=1}^{A} t_{ia} p_{ak} + e_{ik}$$

where x_{ik} is an element of matrix X, i is an index for objects (nuclei), k is an index for variables (image parameters), x_k is the kth element of vector x, t_{ia} is the principal component scores (object related) and p_{ak} is the principal component loadings (variable related), and e_{ik} is the residual for object i and variable k.

On page 1750, Table 1, upper half, Sunblad discloses eleven original parameters which are used to obtain calculated parameters. The lower half of Table 1, identifies the fourteen calculated parameters which Sunblad uses in PCA (as discussed above). Not one of the fourteen parameters is an intensity profile of an image region. The only parameters which are even related to intensity are mean greyvalue, mean absorbance, and their standard deviations. Clearly a "mean" or "average" value is quite the opposite of a profile because it is a constant not a variable. A standard deviation is not a profile either.

The technical superiority of Applicants' invention over Sunblad is seen from the fact that Sunblad requires fourteen parameters to be processed to produce a mitotic index value by a technique which was not fully automated. (See e.g. page 1755, left

column lines 30-31). In contradistinction, Applicants' invention as now claimed in claim 10 requires only one parameter - image profile - to be processed to produce a mitosis measurement by a technique which is automated. Consequently Applicants' invention is different to, simpler and better than Sunblad.

Claims 10, 23 and 31 are further non-obvious because the examiner's motivation for combining the references is technically flawed. The Examiner states that "it would have been obvious to one having ordinary skill in the art at the time the invention was made to modify Vaisberg invention *(sic)* according to the teaching of Sunblad because combination *(sic)* of Vaisberg classifying cells based on information in cell images and Sundbland *(sic)* staining procedure provides improve *(sic)* of measurement of mitotic activity, which can easily be implemented in an imaging device" *(Applicants' italics)*. The examiner's motivation for combining the references is respectfully traversed, because Vaisberg and Sunblad disclose quite different techniques which cannot usefully be combined. Vaisberg images a cell by detecting the cell's DNA, not the cell's structure or nucleus revealed by staining. (See e.g. Vaisberg page 11 lines 32-34). The DNA is induced to emit fluorescence by treating cells with an agent which binds to DNA in the cell only. (See Vaisberg page 11 lines 10-19). In contradistinction, Sunblad images a cell's structure as revealed by staining a cell's nucleus, not its DNA. (See e.g. page 1749, right column, line 21 to page 1750, left column, line 15). The combination of Vaisberg with Sunblad is inoperable if the Examiner's intention is that Sunblad's staining technique replaces the Vaisberg agent inducing fluorescent DNA, because this makes the DNA undetectable. If Sunblad's staining technique does not replace the Vaisberg agent, i.e. if both staining and agent are used together, Sunblad's staining technique is completely redundant and might moreover be counterproductive by affecting the chemical bonding between the agent and DNA or by absorbing or eradicating the fluorescence Vaisberg needs to detect;

In addition, claims 10, 23, and 31 are non-obvious because there is no *prima facie* case of obviousness. Neither Vaisberg nor Sunblad discloses a threshold to discriminate between mitotic and non-mitotic cells. They both use a threshold to discriminate between background and cell (DNA) images (Vaisberg) or nuclei (Sunblad), i.e. between non-cells and cells. (See Vaisberg page 5 lines 7-9 and

Sunblad page 1754 left column last paragraph). There is alternatively no *prima facie* case of obviousness because neither Vaisberg nor Sunblad discloses measuring mitosis from image region intensity profiles by counting an image region as mitotic if its intensity profile has an above-threshold number of pixels with intensity associated with mitotic figure imagery, unlike Applicant's invention as claimed in claim 10. Consequently the combination of Vaisberg and Sunblad does not anticipate claim 10 as now amended.

Finally, Applicant's invention as claimed in claim 10 is non-obvious over the combination of Vaisberg and Sunblad because it is unexpectedly technically superior to the prior art in that it determines mitosis from far fewer parameters than Sunblad and uses standard histopathological imagery unlike Vaisberg.

Claim 10 is non-obvious and patentable for the many reasons recited above. The above arguments presented for claim 10 apply equally to demonstrate claims 23 and 31 are non-obvious.

CONCLUSION

All pending claims are believed to be ready for patenting for the reasons recited above. Favorable reconsideration and allowance of all pending claims is, therefore, courteously solicited.

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